Abstract

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Title of diploma thesis: Influence of salmonella lipopolysaccharide on gene transcription and secretion of interferon gamma
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Aim: The aim of this diploma thesis is to evaluate the influence of a type of salmonella lipopolysaccharide on the activation of gene transcription and production of gamma interferon (IFN-γ) as a key cytokine of immune response to intracellular bacterial pathogens. The study was performed on infectious model of a gnotobiotic pig infected with gram-negative bacteria *Salmonella enterica* serovar Typhimurium strain LT2 or its *rfaL*- mutant.

Methods: 1) Four groups of piglets were created: germ-free as a control group, conventional piglets, piglets infected with LT2 strain, piglets infected with *rfaL*- mutant 2) Determination of bacterial CFU in blood and intestinal lavage by cultivation on bacteriological media 3) Isolation of a total RNA from ileum, spectrophotometric quantification and estimation of its purity (A_{280}/A_{260}) 4) Reverse transcription into cDNA 5) Real-time PCR with LNA probes 6) Normalization and relativization of real-time PCR data 7) Estimation of cytokines in blood and intestinal lavage by ELISA.

Results: 1) A bacterial count in the group infected with LT2 strain outnumbered a count in piglets infected with *rfaL*- mutant 2) The gene transcription of IFN-γ was the highest in the LT2 strain 3) The gene transcriptions of IL-12/23 p40 and IL-18 showed higher levels in LT2 group compare with the control 4) Expression of TLR4 mRNA was upregulated in the group infected with LT2 5) Expression of NF-κB mRNA was increased either in LT2 or in *rfaL*- groups 6) IFN-γ on a protein level was increased both in plasma and intestinal lavage of the LT2 infected pigs 7) IL-12/23 p40 was increased in plasma and intestinal lavage in the LT2 group 8) IL-18 levels differed in plasma and intestinal lavage but the differences among piglets groups were not statistically significant.

Conclusions: The highest activation of mechanisms of innate immunity was observed in the group of gnotobiotic piglets infected with virulent *S*. Typhimirium LT2. The piglets infected with *rfaL*- mutant did not showed symptoms of salmonella enteritidis (anorexia, fatigue, diarrhea, and fever) but the levels of induced cytokines attested about activation of mechanism of innate immunity. It is possible to expect that this Salmonella with R chemotype of the lipopolysaccharide can be effective as a “live vaccine” protecting against infection with virulent strain. However, a thorough verification of such hypothesis requires other complementary experiments of combined application of both *Salmonellas* in experiments *in vivo*.